OPTIMIZATION OF XYLOSE PRODUCTION FROM SUGARCANE BAGASSE USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

Hemicellulose is defined as several polysaccharides that are more complex than a sugar and less complex than cellulose, found in plant cell walls. Xylose is a pentose sugar formed by the hydrolysis of xylan, the substrate found in hemicellulose. Sugarcane bagasse widespread with sugar sources contains about 27% of hemicellulose. After extracting the juice content in sugarcane, the large portion of excess bagasse is burn as waste material causing air pollution. Acid hydrolysis method was used for the pretreatment of sugarcane bagasse to extract hemicellulose compound. The main objective of this research is to optimize the production of xylose from sugarcane bagasse. To achieve the objective, this research was conducted in identified scopes. The scopes of this research includes the study of the effect of agitation time, pH and substrate concentration on xylose production using sugarcane bagasse and to optimize the production of xylose using Response Surface Methodology (RSM). Response Surface Methodology (RSM) is a method in Design Expert software which uses mathematical technique to optimize production based on different parameters. After the pretreatment, hemicellulose was obtained and used to produce xylose using enzymatic hydrolysis method. At the end of this research, it was found that the optimized xylose production from sugarcane bagasse was obtained at agitation time of 5.36 hour, pH 8.73 and substrate concentration of 70.6mg/ml which produce 19.64 mg/ml of xylose. Before optimization, the xylose production was only 13.22 mg/ml and the production of xylose was increased by 49% after optimization.

ABSTRAK

Hemisellulosa didefinasikan sebagai polisakarida yang lebih kompleks daripada gula kurang kompleks berbanding sellulosa. Xilosa adalah gula pentosa yang terhasil daripada hidrolisis xylan, substrat yang terdapat dalam hemisellulosa. Hampas tebu mengandungi kandungan hemisellulosa sebanyak 27%. Biasanya, selepas mengekstrak kandungan air gula dari tebu, hampas tebu yang banyak dibakar sebagai sisa mentah yang menyebabkan pencemaran udara. Objektif utama kajian ini adalah untuk mengoptimumkan penghasilan gula xylosa daripada hampas tebu. Bagi mencapai objektif ini, kajian ini dijalankan berdasarkan skop yang dikenalpasti. Skop kajian ini termasuk kajian masa, pH dan kepekatan substrat terhadap pengnghasilkan xylosa daripada hampas tebu dan untuk mengoptimumkan penghasilkan xylosa menggunakan Kaedah Permukaan Tindakbalas (RSM). RSM adalah kaedah didalm perisian Design Expert yang menggunakan teknik pengiraan matematik bagi mengoptimumkan penghasilan berdasarkan kepada parameter yang berlainan. Selepas dirawat, hemisellulosa digunakan untuk menghasilkan xilosa dengan menggunakan kaedah hidrolisis enzim. Pada penghujung kajian, didapati bahawa penghasilan xilosa daripada hampas tebu yang dioptimumkan dicapai pada masa 5.36 jam, nilai pH 8 and kepekatan substrat 70.6mg/ml yang menghasilkan xilosa sebanyak 19.64 mg/ml. Sebelum pengoptimuman, penghasilan xilosa adalah hanya 13.22 mg/ml dan penghasilan telah meningkat sebanyak 49% selepas pengoptimuman.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of variance
CCD	-	Central composite design
g	-	Gram
g/L	-	Gram per litre
hr	-	Hour
L	-	Litre
M	-	Molar
mg	-	Miligram
min	-	Minutes
ml	-	Mililitre
mM	-	Milimolar
OD	-	Optical density
OFAT	-	One factor at time method
RSM	-	Response surface methodology
rpm	-	Round per minute
T	-	Temperature
U	-	Unit (enzyme activity)
˚C	-	Degree Celsius
%	-	Percentage

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lignocellulosic biomass is one of the most available and renewable resources which represent a promising low cost raw material for the production of biofuel, bioenergy and added value biomolecules. Hemicellulosic materials such as agricultural residues (sugarcane bagasse) offer this possibility (Boussarsar, 2009). Lignocellulosic feedstock is considered as an attractive raw material not only for the liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based because of its availability in large quantities. Corn stover, wheat straw, sugar begasse, rice straw, rice hull, corn cob, oat hull, corn fiber, woodchip and cotton stalk have attracted the most interest of research (Gray *et a*l., 2006). The major chemical components of lignocellulosic biomass lignocellulosic biomass are cellulose, hemicellulose and lignin. Cellulose is a linear polymer of anhydroglucopyranose units linked by ether bonds. Hemicellulose as cellulose, are polymers constituted of sugar units. They differ from cellulose by being smaller and branched polymers usually containing more than one sugar type; they are also amorphous polysaccharides. Lignin is a complex, crosslinked, three-dimensional polymer formed with phenylpropane units (Marion *et al*., 2010).

Nowadays about 50% of generated sugarcane bagasse is used to generate heat and power to run the sugar mills and ethanol plants. The remaining portion is usually stockpiled. However, because the heating value of carbohydrates is approximately half of that lignin, it would be beneficial to develop a more economical use of carbohydrates. One such possibility would be to extract the hemicelluloses before cellulose to convert them to higher value-added products such as prebiotic xylooligosaccharides or polymers and composites for chemical and pharmaceutical applications (Brienzo *et al*., 2009). Agricultural residues, such as sugarcane bagasse contain about 25% of hemicellulose (Roberto *et al*., 2003). The increasing interest in biotechnological processes employing lignocellulosic residues is quite justifiable because these materials are cheap, renewable and widespread sugar sources. Hemicellulosic hydrolysates made from such residues have been frequently utilized instudies for developing a technically and economically viable bioprocess (Roberto *et al*., 2003).

Among biomass components, hemicelluloses which are mainly composed of xylans, provide an important source of interesting molecules such as xylose and xylo-oligosaccharides which have potential applications in different areas, notably in chemical, food and pharmaceutical industries (Boussarsar, *et al* 2009). The quantity and quality of hemicelluloses in the crops are quite variable and depend on the considered species. They are usually defined as polymers that are solubilized from plant cell walls by alkali. There are biopolymers of a limited number of sugars, mainly the xylose, mannose, glucose, galactose, arabinose, acid glucuronic. Xylose is always the sugar monomer present in the largest amount in hemicellulose (Derriche *et al*., 2007). A hemicellulose can be any of several heteropolymers (matrix polysaccharides), present in almost all plant cell walls along with cellulose. Hemicellulose has a random, amorphous structure with little strength. Therefore it is easily hydrolyzed by dilute acid or base (Rahman *et al*., 2006).

Xylose is the second most abundant sugar in lignocellulosic materials after glucose and its efficient conversion is one of the prerequisites for lignocellulosic industrialization. Xylans, a major wood cell wall hemicellulose with a β-1,4-linked xylopyranosyl backbone, comprise up to 35% of hardwood and 14% of softwood (Ishihara *et al*., 2002) Xylose, is a monosaccharide containing five carbon atoms and including an aldehyde functional group. It has chemical formula $C_5H_{10}O_5$. Xylose is found in the embryos of most edible plants. With its free carbonyl group, it is a reducing sugar. This xylose can be used as a substrate to produce a wide variety of compounds or fuels by chemical or biotechnological processes.

Reduction of xylose by catalytic hydrogenation produces the sugar substitute xylitol. Xylitol is a polyol whose properties have attracted the attention of at least three types of industries: food (for its sweetening power and insulin-independent metabolism), odontological (for its anticariogenicity, tooth rehardening and remineralization properties) and pharmaceutical (for its capability of preventing otitis and its possibility of being used as a sweetener or excipient in syrups, tonics and vitamin formulations) (Roberto *et al*., 2003).

The cellulose and hemicellulose content of sugarcane bagasse can be hydrolyzed chemically or enzymatically to obtain reducing sugar such as xylose. Dilute-sulfuric acid hydrolysis is a chemical hydrolysis for either the pretreatment before enzymatic hydrolysis or the conversion of lignocellulose to the corresponding sugars (Taherzadeh *et al*., 1997). In dilute-acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulose fraction. If higher temperature or longer retention times are applied, the formed monosaccharides will be further hydrolyzed to other compounds. It is therefore suggested that the hydrolysis process be carried out in at least two stages, the first stage at relatively milder conditions during which the hemicellulose fraction is hydrolyzed and a second stage can be carried out by enzymatic hydrolysis or dilute acid hydrolysis at higher temperatures during which the cellulose is hydrolyzed (Karimi *et al*., 2006).

Roberto *et al*., (2003) stated the optimization of xylose production from oil palm empty fruit. The objective of the study was to determine the effects of H_2SO_4 concentration and reaction time on the production of sugars (xylose, glucose and arabinose) and on the reaction byproducts (furfural, hydroxymethylfurfural (HMF) and acetic acid). Dilute sulfuric acid was used as a catalyst for the hydrolysis of rice straw at 121 °C hydrolysis reactor. Rationale for conducting this study was determined based on a central composite statistical design. Response surface methodology (RSM) was adopted to optimize the hydrolysis conditions aiming to attain high xylose selectivity. The optimum H_2SO_4 concentration of 1% and reaction time of 27 min was found. Under these conditions, 77% of xylose yield and 5.0 g g^{-1} of selectivity were attained.

1.2 Problem Statement

The amount of organic waste obtained from the agriculture industry is abundant in Malaysia but the utilization is still limited. Sugarcane (*Saccarhum officinarum*) bagasse is a residue produced in large quantities by sugar industries. In general, 1 tonne of sugarcane bagasse generates 280 kg of bagasse, the fibrous by-product remaining after sugar extraction from sugarcane. Since bagasse is a by-product of the sugarcane industry, the quantity of production in each country is in line with the quantity of sugarcane produced. However, the utilization of sugarcane bagasse is still limited and is mainly used as a fuel to power the sugar mill. When burned in quantity, it produces sufficient heat energy to supply all the needs of a typical sugar mill, with energy to spare. This resulting CO_2 emission to the atmosphere which causes environmental pollution. (Sun *et al*., 2004).

After extracting the juice content in sugarcane, the large portion of excess bagasse is burn as waste material. Field burning is the major practice for removing sugarcane bagasse, which increases the air pollution and consequently affects the public health. But Sugarcane bagasse mainly contains cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%) which can utilize for various commercial purposes, especially the demand of xylose from hemicellulose increases in many industries (Brienzo *et al*., 2009). Therefore this research is aimed to optimize the xylose production using sugarcane bagasse.

1.3 Objectives

The main objective of this research is to optimize the production of xylose from sugarcane bagasse using Response Surface Methodology (RSM).

1.4 Scopes of Research

The scopes of study on the production of xylose are:

i. To study the effect of agitation time on xylose production from sugarcane bagasse

ii. To study the effect of pH on xylose production from sugarcane bagasse.

iii. To study the effect of agitation time on xylose production from sugarcane bagasse.

iv. To optimize the production of xylose from sugarcane bagasse using Response Surface Methodology (RSM).

1.5 Rationale and Significance

Malaysia is one of the major country which produces sugarcane every year. In 2009 the total amount of sugarcane production is 755,770 metric tons which consist of 13,880 hectar. From year 2007 to 2012, there will be about 18 percent in excess of the area needed for domestic sugar self-sufficiency, to an aggregate hectare that will supply feedstock for both sugar starting from 2008 as needed, without affecting sugar self-sufficiency (Charles *et al.*, 2003). The large amount of sugarcane bagasse should be utilized in beneficial way rather than burning it. By doing this, environmental pollution can be minimized.

Response Surface Methodology (RSM) will provide the optimal condition to increase the xylose production from sugarcane bagasse. By regulating different parameters that affect the production of xylose, the optimization of the xylose from sugarcane bagasse can be obtained effectively. The advantage of RSM is it is fast, can investigate the interaction between parameters as well as increase the production rate of xylose, thus fulfill the commercial demand (Psomas *et al.*, 2007).

CHAPTER 2

LITERATURE REVIEW

2.1 Xylose

Xylose can be use as substrate to produce a wide variety of compounds or fuel by chemical or biotechnological processes. With sustained hydrolysis xylose may be degraded to such decomposition products as furfural, hydroxymethylfurfural and furan resins (Lavarack *et a*l., 2002). Xylose can also be hydrolyzed from xylan-rich materials like rice husk, corn stalk, wheat straw and flax straw. Xylose is hydrogenated at high pressure to xylitol by using a nickel catalyst. (Sjöman *et al*., 2008). In health perspective, xylitol can be a better alternative for people with diabetes because it does not raise insulin level in human body. Xylitol is a sugar alcohol having sweetness equal to sucrose but it does not cause dental carries and thus it is used as a sweetener by the confectionary industry. Xylose is also a versatile sugar compound and has many applications such as sugar source for non-nutritive agent in pharmaceutical industry, additive in colour photography and brightener in zinc electroplating (Murthy *et al*., 2005). Potential sources for xylose are birch and other hardwoods that have a xylan rich hemicellulose structure. In chemical wood pulping processes hemicelluloses are hydrolyzed and xylose is found in rejected spent liquor (Alen *et al*., 2000).

2.2 Hemicellulose

Hemicelluloses are known as valuable in pulp additives, natural barrier for packaging films and as components of skin substitutes in case of damage of superficial epidermal layers. As hemicelluloses are relatively tightly bound in the plant cell wall network to lignin and cellulose, it is difficult to separate them without significant modification of their structure. Different treatments have been applied to hemicellulose extraction. Acid hydrolysis and hydrothermal methods are usually much preferable (Brienzo *et al*., 2009). In addition, wood-based hemicellulose hydrolyzates contain lignosulfonates and inorganic ions (Sjöman *et al*., 2008). There are several xylose purification operation from hemicellulose hydrolyzate, acid hydrolysis, enzymatic hydrolysis to produce xylose which could partially improve purity and yield in commercial xylose production. In several plants the majority of hemicelluloses is xylan which can be hydrolyzed into xylose. Particularly the hemicellulose of hardwood is rich in xylan. Consequently it is possible to obtain xylan and xylose as by-products from cellulose industry using hardwood. (Gunda *et al.,* 1970)

2.3 Hemicellulose Conversion to Simple Sugar

Different treatments have been applied to hemicellulose extraction and heat treatment is often combined with addition of chemicals such as alkali, acid or hydrogen peroxide. In order to obtain fast enzymatic hydrolysis of biomass with a high sugar yield (for both hexoses and pentoses), the two main protective coats around cellulose, hemicellulose and lignin need to be removed or altered without degrading the hemicellulose sugars. Hemicellulose forms a physical barrier around the cellulose (Öhgren *et al.,* 2006). Acid hydrolysis is an effective agent for both delignification and solubilization of hemicelluloses. In these conditions carbohydrates are less damaged and delignification is more efficient. However, the use of acid treatment of bagasse requires prior removal heavy metals with chelating agents. The metals catalyze decomposition of the peroxide anion in the alkaline medium leading to the formation of hydroxyl radicals which cause hemicellulose depolymerization and diminish its recovery. However, chelation not only removes heavy metals, but also alkali earth metals which act as natural stabilizers of the peroxide during the treatment . This is the reason why magnesium ions are added later in a surplus to the chelating agents to prevent hemicellulose degradation (Brienzo *et al*., 2009).

According to Neureiter *et al*. (2002), acid concentration is the most important parameter affecting sugar content, while for the formation of sugar degradation products, temperature has the highest impact. The main problem encountered in this process is the generation of a large number of degradation products that can strongly affect the microbial metabolism. To overcome this problem, it is necessary to select satisfactory reaction conditions to keep the degradation products at low levels because their type and levels depend on the severity of the hydrolysis reaction.

2.4 Enzymatic Hydrolysis

Enzymatic hydrolysis is a process in digestion in which macromolecules are split from food by the enzymatic addition of water. Enzymatic hydrolysis can not only economize energy on account of the relatively mild reaction conditions, but also avoid using toxic and corrosive chemicals (Xu *et al*., 2007). Enzymatic hydrolysis of biomass hemicellulose does not produce toxic products. Alternatively, xylose can be produced by enzymatic hydrolysis xylan. Generally, in lignocellulosic biomass, xylan exists in xylan–lignin complex and becomes resistant to hydrolysis (Zhu *et al*., 2006). Therefore xylose production is carried out in two stages: alkaline extraction of xylan from lignocellulosic biomass followed by enzymatic hydrolysis (Akpinar *et al*., 2009).The enzyme hydrolysis is catalyzed by xylanase enzyme for xylose production. The greatest potential for sugar production from biomass also lies in enzymatic hydrolysis of cellulose and hemicellulose using cellulase and hemicellulase enzymes. Although the structure of hemicellulose is more complex than cellulose and requires several different specificities for complete hydrolysis, the polysaccharide does not form tightly packed crystalline structures like cellulose does and thus is more accessible to enzymatic hydrolysis. In hemicellulose, enzymatic hydrolysis requires mild conditions and long periods of time (Saha *et al*., 2004).

From the research done by Rahman *et al*. (2006) it was revealed that under controlled treatment conditions, acid hydrolysis of lignocellulosic biomass mainly produced xylose from xylan with cellulosic and lignin fractions remaining unaltered. The solid residue can further be utilized for production of ethanol or in pulp processing for making high grade paper. In the hydrolysis process it is understood that initially the lignin protective layer around the hemicellulose fiber is softened under elevated temperature and pressure which allows the acid to penetrate the layer and hydrolyze the amorphous xylan to form xylose. On the other hand the condition is not severe enough to hydrolyze the crystalline structure of cellulose which remains as insoluble solid. Although xylose was the main sugar obtained from hemicellulose, other byproducts such as glucose, acetic acid, furfural, etc. were also produced in low amount during the hydrolysis process.

2.5 Factors Affecting the Hemicelluloses Degradation

2.5.1 Effect of Agitation Time

Time is one of the most important factor in enzymatic hydrolysis process. Generally, production rate of reducing sugar increases as time increases. But after a certain time period the production rate will start to decrease. It is speculated that a reduction in the reaction rate may be due to the limitation of the enzyme activity by formation of reaction products at high degrees of hydrolysis as time increases.

Roberto *et al*. (2006) investigated the conversion of rice straw into reducing sugar (xylose) in a semi-pilot reactor Hydrolysis of rice straw by dilute sulfuric acid at different time were investigated. The hydrolysis method was carried out in a 350-L reactor with pressure (35 bar) and acid concentration (1.25%) at different time (0 – 50 min). The results show the ability of first stage hydrolysis to depolymerize xylan to xylose with a maximum yield of 77% at 180min.

According to Xu *et al*. (2007) the pretreated rice straw was hydrolyzed using cellulase in stoppered Erlenmeyer flasks to determine the optimal agitation time. The hydrolysis was performed in 0.1 M citrate buffer (pH 4.8) at 150 rpm at 50 °C under shaking. To determine the effectiveness of time on enzymatic hydrolysis, enzyme to substrate ratio was maintained at 30 FPU/g of substrate. The experiment was conducted at hydrolysis time (6 – 48 h) on enzymatic hydrolysis of soybean straw. The highest yield of xylose obtained at 36hr with the recovery of 51.22%.

2.5.2 Effect of pH

pH has strong effect on hemicellulose degradation to produce reducing sugar (xylose). Generally, the yield of xylose produced is higher at pH range of 3-6. According to Zabihi *et al* (2010), the hydrolysis rate for the production of xylose from wheat straw is higher at pH 4.8 with temperature (50 °C) and agitation speed (240 rpm).

Chen *et al*. (2010) investigated the optimum pH level for xylose production. The enzymatic saccharification of hemicellulose in dried residue after lime treatment or bio-degradation was performed by shaking gently (120 rpm) at 50 °C in 250 mL Erlenmeyer flask containing buffer H3PO4. The substrate content for reaction was 10.0% (w/v) and Tween-80 as surfactant (1.0%, v/v) was used. Before enzyme loading, slurry was acclimated by incubating at 50 °C on a rotatory shaker (ZHWY-211B, Tocan Scientific, Shanghai, China) at 120 rpm for 30 min. At end of the research it is found that production of xylose is higher at pH 5.3 with the production rate of 29.87mg/ml.

Martinez *et al*. (2003) also investigated the influence of pH on continuous production of xylose from sugarcane bagasse hemicellulosic hydrolysate by *C. guilliermondii*. Experiments were carried out in a reactor with 1.25 l of treated hydrolysate, at 30 °C and 300 rpm using different pH level (4.0 – 7.0). It is found that the yield of xylose concentration from sugarcane bagasse hemicellulosic hydrolysate at pH level of 7.

2.5.3 Effect of Substrate Concentration

The substrate concentration is another variable that influenced the xylose yield and xylose conversion during the enzymatic hydrolysis. After reaching the optimal condition, the yield of reducing sugar starts to fall**.** Such effect can be attributed to end-product inhibition caused by high concentration of substrate, and mass transfer limitations within the reaction mixture due to the high viscosity of the slurry. Other factors that can contribute to the low degree of carbohydrate conversion at high substrate concentration, mainly when low enzyme loadings are employed, include the decrease in the reactivity of cellulosic material in the course of hydrolysis.

Xu *et al*. (2007), conducted studies on enzymatic hydrolysis of pretreated soybean straw. The effect of substrate concentration (2–20% w/v) on xylose production together with the effects of other parameters was investigated. The hydrolysis rate increased up to substrate concentration of 5%. Maximum hydrolysis rate of 43.73% was achieved at substrate concentration of 5%. Further increase in the substrate concentration decelerated the rate of hydrolysis.

2.6 Response Surface Methodology

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful developing, improving and optimization processes. The most extensive applications of RSM are in the particular situations where several input variables potentially influence some performance measure or quality characteristic of the process. Central composite design is a type of response surface methodology. It is a general linear model in which attention is focused on characteristics of the fit response function, in particular, where optimum response value is occur. The yield data were analyzed for model fit using the RSM software (Design Expert) (Corredor *et* al., 2006).

Response surface methodology (RSM) uses quantitative data from appropriate experiments to determine and simultaneously solve multivariate equations. It is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and analyzing optimum conditions of factors for desirable responses. It has been successfully utilized to optimize compositions of fermentation medium, conditions of enzymatic hydrolysis, synthesis parameters for polymers and parameters for food processes (Li *et al*., 2006).

A successive response surface method is an iterative method which consists of a scheme to assure the convergence of an optimization process. The scheme determines the location and size of each successive region of interest in the design space, builds a response surface in this region, conducts a design optimization and will check the tolerances on the response and design variables for termination. This RSM method has been widely used to evaluate and understand the interaction between different physiological and nutritional parameters (Hounjg *et al.,* 1989)

Psomas *et al*. (2007) stated that the response surface methodology (RSM) was applied to optimize the cultural conditions for xanthan gum production by *Xanthomonas campestris*, to maximize cell and xanthan production in batch experiments using a synthetic broth (Luria-Bertani plus glucose, LBG). The interactive effects of three independent variables (agitation rate (100–600 rpm), temperature (25–35 °C), time of cultivation (24–72 h) on xanthan gum and were studied. A second ordered polynomial model was fitted and optimum conditions were estimated. At end of the research the optimal xanthan gum production was found at 600 rpm 30 °C at 72 h and biomass at 600 rpm, 25 °C at 72 h.

Cruz *et al*. (2010) conducted a research aimed to optimize probiotic yogurt containing glucose using Response Surface Methodology (RSM) and to determine the levels of glucose and glucose oxidase that minimize the concentration of dissolved oxygen and maximize the *Bifidobacterium longum* count by the desirability function. RSM mathematical models adequately described the process, with adjusted determination coefficients of 83% for the oxygen and 94% for the *B. longum*. The desirability function indicated that 62.32 ppm of glucose oxidase and 4.35 ppm of glucose was the best combination of these components for optimization of probiotic yogurt processing.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, several methods were taken to investigate the effect of different parameters for the production of xylose from sugarcane bagasse. First, acid hydrolysis method used for pretreatment to delignified the sugarcane bagasse. Next, the pretreated sugarcane bagasse undergoes enzymatic hydrolysis method to obtain xylose. Then based on results obtained from one factor at a time method (OFAT), optimization for the xylose production was carried by using Response Surface Methodology (RSM). Experiment was conducted according the data that obtained from Response Surface Methodology. Figure 3.1 shows the overall flow of xylose production from sugarcane bagasse.